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In Silico Investigations on Basal Stem Rot Disease and Biocontrol in *Elaeis Guineensis*

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Abstract: Palm oil is the main vegetable oil produced in Nigeria and *Elaeis guineensis's* health is crucial in obtaining maximum production of oil. The genus Ganoderma belongs to the family of Ganodermataceae, which causes white rots of hardwoods in many woody plants like *E. guineensis* by decomposing their lignin with peroxidase and laccase. Introducing endophytic bacteria to the roots of E. guineensis could lead to suppression in the growth of Ganoderma specie. This study investigated microbial natural biocides capable of controlling basal stem rot disease in *E. guineensis*. Homology modeling of ganodermal manganese dependent peroxidase and laccase was achieved with ModWeb online tool. Secondary metabolites were manually curated and lignin was modeled as guaiacol. Virtual screening was achieved with AutoDockVina® on Linux platform. The result showed that eight (8) compounds had better binding affinities when compared with guaiacol. Catechin and flavanone are lead secondary metabolites with biocontrol potential against G. lucidium and binds to the same site that guaiacol binds on ganodermal manganese dependent peroxidase and laccase while flavanone binds to a different site on laccase. Stem rot disease in E. guineensis could be better controlled with natural biocides in endophytic bacteria such as catechin and flavanone.

Keywords: *Elaeis Guineensis*, Catechin, Flavanone, Lignin, Laccase, Manganese Peroxidase, *G. Lucidium*, Bio-Control, Natural Biocides

1. Introduction

Palm oil is the main vegetable oil produced in Nigeria. Its output indeed represented only 3% of the world production in 2010 [1]. As regards to palm oil production in Africa, however, Nigeria's production is estimated at 55% of the African output [1]. Plant health is crucial in obtaining maximum production. The genus *Ganoderma* belongs to the family of *Ganodermataceae*, which causes white rots of hardwoods in many woody plants by decomposing lignin as well as cellulose and related polysaccharides [2]. *Ganoderma* spp. is an economically important plant pathogen especially in oil palm, causing basal stem rot disease. The disease progresses slowly, but eventually all infected plants become dead [3]. The *Ganoderma spp*. can infect oil palm at all stages, from seedlings to old plants. Basal stem rot (BSR) disease in oil palm is adversely found in Malaysia and Indonesia and in other South East Asian countries, also included are Papua New Guinea, Ghana, Nigeria, Africa, Central America, Cameroon, Tanzania, Zimbabwe, and Thailand [4] Angola, North Rhodesia, Zaire [2, 5].

G. lucidum, a white-rot *Basidiomycetes* is widely distributed worldwide and grows predominantly on deciduous trees and rarely on coniferous trees [6, 7]. White-rot fungi are capable of degrading all basic wood polymers, due to their ability to syntheses relevant hydrolytic and oxidative extracellular enzymes. This can lead to economic loss of oil palm [8]. These enzymes are responsible for the degradation of cellulose, hemicellulose and lignin into low-molecular-weight compounds that can be assimilated for fungi nutrition [9]. The enzymes produced by *Ganoderma* species as white-rot ones are: lignin peroxidases (LiP), Mn-oxidizing peroxidases (Mn-dependent peroxidases (MnP), versatile peroxidases (VP)), and laccase (Lac).

Field control of BSR by contact chemicals have not been very successful [10]. Biological control need not necessarily be a cure for the disease and can be merely to arrest the disease spread by inoculation with a biocontrol agent. For example, saprophytes can be used to compete against *Ganoderma* to reduce its opportunity for colonizing oil palm. Endophytic bacteria are organisms inhabiting plant organs that at some time in their life cycle can colonize the internal plant tissues without causing apparent harm to the host [11]. Introducing endophytic bacteria to the roots to control plant disease is to manipulate the indigenous bacterial communities of the roots in a manner, which leads to enhanced suppression of soil-born pathogens. The use of endophytic bacteria should thus be preferred to other biological control agents as they are internal colonizers, with better ability to compete within the vascular systems, limiting Ganoderma for both nutrients and space during its proliferation.

Burkholderia species have been isolated from many different environmental niches, including soil and water, and can form associations with plants, animals and humans [12].Several *Burkholderia spp.* are widespread in nature and some of them are considered to be beneficial in the natural environment [12]. *Burkholderia* species are widely distributed in natural environment being present in the soil, water and plant rhizosphere. Customarily, *Burkholderia* species are plant pathogens, but some have been studied as biocontrol agent of plant diseases. Particularly, among these, strains of *B. gladioli* possess a great potentiality as plant pathogen antagonists.

Several bacteria species including some Pseudomonas and *Burkholderia* produce natural bioactive secondary metabolites such as Pyrrolnitrin and Pyoluteorin [13]. Recently, it was demonstrated that volatile organic compounds (VOCs) of bacteria such as terpenoids, phenylpropanoids and fatty acid derivatives can influence the growth of some fungi through their inter- and intra-organismic communication signals [13]. Indeed many *Burkholderia spp.* have the ability to produce secondary metabolites with relevant biological activities which can serve as biopesticides [14]. The need to search for microbial natural biocides that will not only control basal stem rot disease but also protect our environment in order to avoid environmental pollution from use of synthetic pesticides informed the decision of the present study.

2. Materials and Methods

2.1. Homology Modeling

Because the 3-D coordinate of laccase and manganese-dependent peroxidase from *Ganodema* specie were not available in pdb database as at the time of the investigation, comparative homology modeling of their structures were achieved with their amino acid sequence obtained from GenBank (Accession numbers: AAR82930.1 and ACD44889.1 respectively) using ModWeb Server [14]. Briefly, one amino acid sequence was submitted and a total of 152 and 81 hits were detected for laccase and manganese-dependent peroxidase respectively. Number of models due to the sequence were calculated and three best models for each of the enzymes were selected from the calculated models using ModPipe quality score (MPQS), Estimated native overlap (TSVMOD), Discrete optimized protein energy (DOPE) and LONGEST_DOPE as selection criteria.

The reliability of the models were evaluated using MPQS $\geq$ 1.1, TSVMod No. 35 (estimated native overlap at 3.5 Å) $\geq$ 40 %, GA341 (Model score) $\geq$ 0.7, E-value < 0.0001 and zDOPE< 0 as evaluation criteria [15]. Therefore, the reliability/fold assignments of the selected models were evaluated using the above mentioned criteria. On this basis, one of the models (model 1) was selected and further evaluated with ModEval Server [16]. Briefly, amino acid sequence and 3-d coordinate of the selected model was submitted to ModEval Server and model evaluation parameters (Predicted RMSD, Predicted native overlap, sequence identity, zDOPE, GA341, z-pair, z-surf, z-combi and DOPE profile) were calculated and returned.

2.2. Visualization of Surface Cavities

Visualization of the surface cavities of laccase and Mn-peroxidase was achieved with UCSF Chimera 1.9 [17]. Minimum, medium and maximum cavity scores were assigned light blue, green, and maroon respectively while cysteine residues on surface were marked yellow. Also, the volume and area of main surface cavity in the laccase and Mn-peroxidase and their templates (2qt6A and 4bm1A respectively) were calculated with UCSF Chimera 1.9.

2.3. Loop Modeling/Optimization

Comparative protein structure prediction is limited mostly by the errors in alignment and loop modeling [18]. In order to obtain quality models, loops were modeled locally with Modeller 9.14 using DOPE modeling protocol [19, 20]. Briefly, the 3-D coordinate of the protein was used, DOPE modeling protocol was specified and Modeller 9.14 was called from UCSF Chimera 1.9 interface to generate a total of five (5) models. The results were analyzed with UCSF Chimera 1.9 and the model with lowest zDOPE score was used for virtual screening.

2.4. Virtual Screening

2.4.1. Preparation of Receptor

AutoDock-vina® [21] was employed to gain insight into the ligand binding to laccase and Mn-peroxidase. Both laccase and Mn-peroxidase were prepared for docking simulation using MGLTools-1.5.6 [22, 23]. Briefly, both proteins were treated as rigid molecules, polar hydrogens only were added, Grid box sizes of 45 x 45 x 45 Å and center of 13.261 x 0.844 x 44.224 at 1.0 Å was applied to laccase while grid box sizes of 40 x 40 x 45 Å and center of -1.05 x 0.158 x 3.706 at 1.0 Å was applied to Mn-peroxidase using MGLTools-1.5.6 [22, 23]. Exhaustiveness of 12 was applied to both systems.

2.4.2. Preparation of Ligands

Guaiacol, a model compound for lignin [25] and some secondary metabolites (caryophyllene, elemene, humulene, caryophyllene oxide, d-limonene, camphene, flavanone, beta-bourbonene, pinene, pyrrolnitrin, phenazine, pyoluteorin, p-coumaric acid, catechin and caffeic acid) were manually curated [13-14, 26-27]. The 3-D coordinates of the curated molecules were obtained from ZINC® Database [28] and prepared for docking simulation using MGLTools-1.5.6 [23, 24] Briefly, all hydrogens were added and rotatable bonds were assigned for the ligands. The prepared receptors (laccase and Mn-peroxidase) and the ligands (guaicol and the secondary metabolites) were used for molecular docking simulation.

2.5. Molecular Docking Simulation and Data Analysis

AutoDockvina® has reported high accuracy in predicting binding free energies by setting the receptor rigid while appraising flexible ligands with a comparatively low standard error [21, 29]. Therefore, lacase and Mn-peroxidase conformational flexibility were neglected by rigid receptor docking. The ligands were docked into lacase and Mn-peroxidase using AutoDockvina® and the virtual screening was done in quadruplet on a Linux Platform. The binding affinities were calculated and reported as mean ± SD. The ligands were ranked according to their binding affinities for laccase and Mn-peroxidase and compared with guaiacol. Visualization and interaction analysis was achieved with MGLTools-1.5.6 while structural alignment of the laccase and Mn-peroxidase models and their templates was done with Pymol-1.4.2.

3. Results and Discussion

3.1. Comparative Homology Modeling

One sequence each was submitted for manganese peroxidase and laccase and 81 and 152 hits were detected for manganese peroxidase and laccase respectively. Also, 47 and 102 models were calculated for manganese peroxidase and laccase respectively. The result showed that the *G. lucidium* models of manganese peroxidase and laccase are homologs of laccase from *Lentinus tigrinus* and manganese peroxidase 4 from *Pleurotus ostreatus* respectively. Their sequence identities are 62.00 % and 72.00 % respectively (Table 1). Their structural alignments also show grate similarities (Figure 1).

Table 1. *Homology modeling parameters of G. lucidium manganese peroxidase and laccase.*

Parameters	Manganese peroxidase			Laccase		
	Model 1	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**
Target region	27-361	26-361	23-361	22-521	22-521	22-521
Protein length	364	364	364	521	521	521
Template pdb code	4bm1A	2e39A	1b80A	2qt6A	2hrgA	3divA
Template region	2-336	9-344	-3.339	1-498	1-496	1-499
Sequence identity	62.00 %	54.00%	53.00 %	72.00 %	70.00 %	69.00 %
E-value	0.00	0.00	0.00	0.00	0.00	0.00
GA341	1.00	1.00	1.00	1.00	1.00	1.00
MPQS	1.74473	1.65838	1.63572	1.91909	1.90109	1.88609
z-DOPE	-1.14	-1.05	-0.94	-1.52	-1.54	-1.49
TSVMod Method	MSALL	MTALL	MTALL	MTALL	MTALL	MSALL
TSVMod RMSD	1.773	1.34	1.177	0.85	0.898	0.879
TSVMod NO 35	0.947	0.965	0.958	0.978	0.978	0.966

Based on the selection criteria in table 1, model 1 of Mn-peroxidase and laccase were selected for further evaluation.

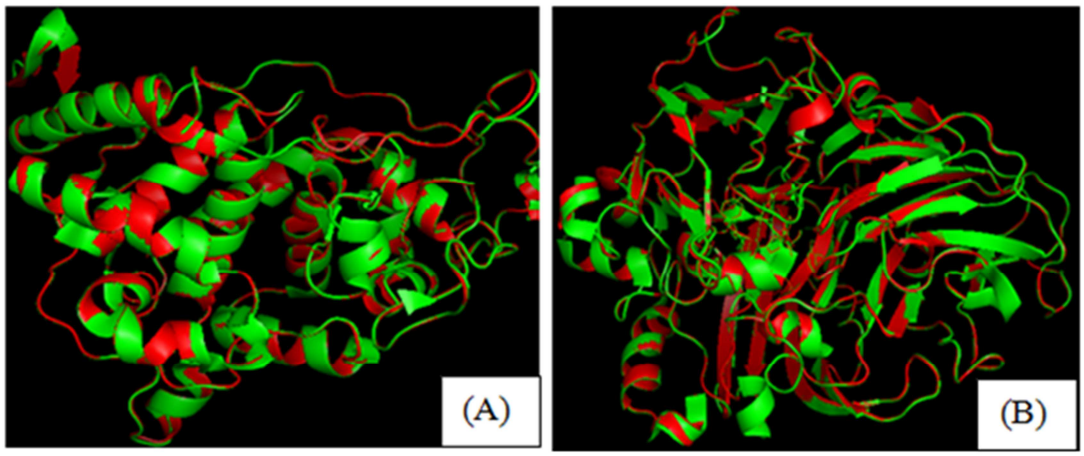

Figure 1. *Structural alignment of Model 1 of (A) Manganese dependent peroxidase and its model template (4bm1A) and (B) laccase and its model template (2qt6A). The templates are coloured green while models of manganese dependent peroxidase and laccase from Ganodema lucidium are colored red.*

It was also observed that the manganese peroxidase and laccase showed a main surface volume of 37.86e3 and 60.00e^3 and area of 13.67e^3 and 18.83e^3 respectively. Also, the models showed a minimum cavity score (light blue), medium cavities score (green), but have no cysteine residues on surface (Figure 2).

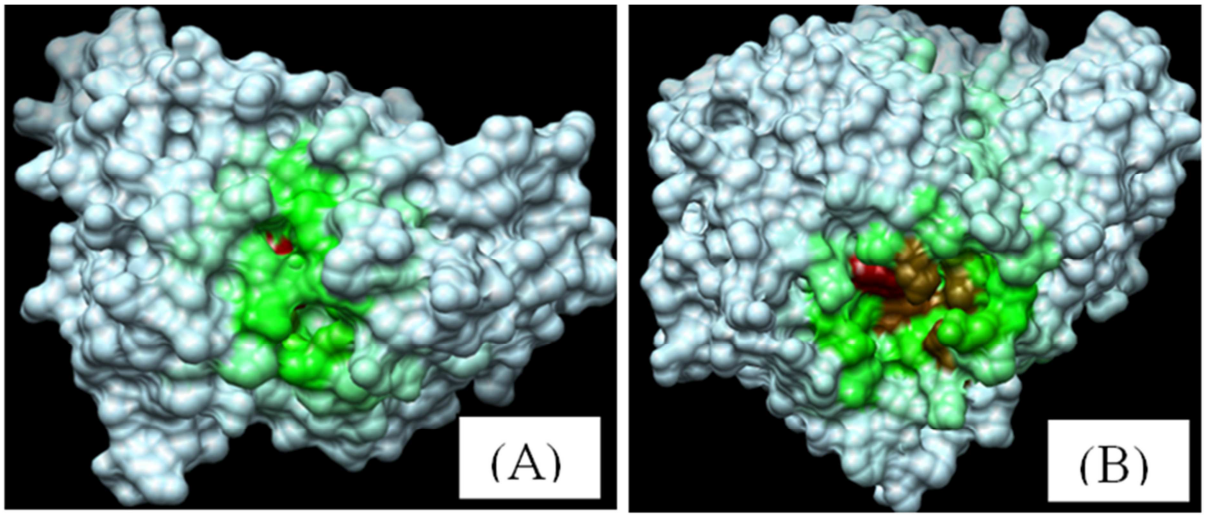

Figure 2. *Cavities of best models of (A) Mn-peroxidase and (B) laccase.*

3.1.1. Model Evaluation

Table 2 showed the TSVMod and Modeller scoring results of Mn-peroxidase and laccase while figure 4 showed the DOPE profile of Mn-peroxidase and laccase. The result showed that *G. lucidium* model of Mn-peroxidase and laccase were reliable (have a reliable fold assignment) because it showed MPQS ≥ 1.1, TSVMod No. 35 (estimated native overlap at 3.5 Å) ≥ 40 %, GA341 (Model score) ≥ 0.7, E-value < 0.0001 and zDOPE < 0 [15]

Table 2. *Model evaluation parameters of G. lucidiumMn-peroxidase and laccase.*

TSVMod Results	Mn-peroxidase	Laccase	Modeller Scoring Results	Mn-peroxidase	Laccase
Match Type	MatchBySS	MatchBySS	Sequence identity	61.791	72.490
Features used	Reduced	Reduced	z-DOPE	-1.114	-1.522
Relax count	1	1	GA341	1.000	1.000
Set size	311	186	z-pair	-9.632	-11.027
Predicted RMSD	2.168	0.647	z-surf	-5.948	-6.512
Predicted Native Overlap (3.5)	0.934	0.966	z-combi	-11.554	-13.082

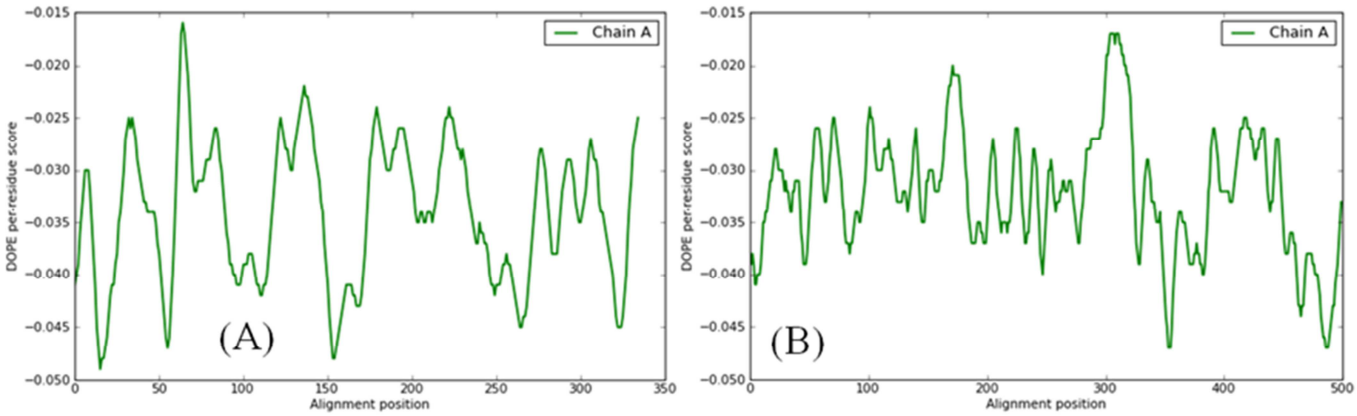

Figure 3. *DOPE profile of (A) Mn-peroxidase and (B) Laccase.*

3.1.2. Loop Optimization

Comparative protein structure prediction is limited mostly by the errors in alignment and loop modeling [18]. Therefore, better quality loops in laccase was achieved at the expense of more computer time by using DOPE-based loop modeling protocol [20]. Loop was not observed in Mn-peroxidase model. The result showed that model 1 had a better quality loop compared to other models because it has the lowest zDOPE (Table 3).

Table 3. *Loop modeling of G. lucidium laccase.*

Model	GA341	zDOPE
1	1.00	-1.13
2	1.00	-1.11
3	1.00	-0.74
4	1.00	-1.01
5	1.00	-0.81

3.2. Molecular Docking Simulation

3.2.1. Binding Affinity

The secondary metabolites were ranked according to their mean binding affinities and compared with a model lignin compound (Guaiacol). The result showed that eight (8) compounds had better binding affinities when compared with guaiacol (Table 4). It is evident from table 4 that catechin and flavanone are lead secondary metabolites with biocontrol potential against *G. lucidium.* Since *G. lucidium* degrades lignin using Laccase and manganese dependent peroxidase and guaiacol was used as lignin model. Therefore any compound will that effectively counteract the effect of *G. lucidium* will not only have higher binding affinity for Laccase and manganese dependent peroxidase than guaiacol but will also bind to the same binding site where guaiacol binds on Laccase and Mn-peroxidase.

Table 4. *Binding affinities of some secondary metabolites for laccase and manganese peroxidase from Ganodema lucidium.*

Molecules	Zinc code	Lac Affinity (Kcal/mol)	Mn-p Affinity (Kcal/mol)
Catechin	28974985	-8.10±0.20	-10.00±0.0
Flavanone	58114	-8.00±0.00	-9.63±0.05
Catechin	119985	-7.80±0.00	-9.30±0.00
Catechin	119983	-7.78±0.15	-9.60±0.00
Flavanone	58113	-7.70±0.00	-10.13±0.1
Pyoluteorin	1727088	-7.08±0.05	-8.60±0.00
Caryophyllene oxide	2083320	-6.80±0.00	-6.50±0.00
Beta-bourbonene	59064310	-6.75±0.06	-7.18±0.05
Beta-caryophyllene	8234282	-6.70±0.00	-6.75±0.70
Humulene	96015176	-6.60±0.00	-6.40±0.20
Pyrrolnitrin	2012	-6.60±0.00	-7.50±0.14
Guaiacol	13512224	-6.40±0.00	-5.60±0.00
Phenazine	8683005	-6.40±0.00	-8.48±0.05
Beta-bourbonene	59064308	-6.38±0.05	-6.40±0.20
Caffeic acid	58172	-6.35±0.06	-7.40±0.00
Beta-elemene	14096289	-6.25±0.06	-6.93±0.05
Indole-3 acetic acid	83860	-6.23±0.05	-7.60±0.00
Camphene	1673034	-6.00±0.57	-5.20±0.00
P-coumaric_acid	39811	-5.98±0.05	-7.20±0.00
Camphene	968230	-5.90±0.12	-5.18±0.05
Beta-pinene	967582	-5.68±0.05	-5.53±0.01
D-limonene	967513	-5.53±0.05	-6.70±0.00
D-limonene	968226	-5.50±0.08	-6.78±0.05
Beta-pinene	1530385	-5.45±0.24	-5.40±0.12
Alpha-pinene	967580	-5.33±0.05	-5.50±0.00

3.2.2. Analysis of the Binding Sites of the Best Poses

To understand whether catechin and flavanone bind to the same site that guaiacol binds, there binding sites were visualized and compared. The result showed that catechin and flavanone binds to the same site that guaiacol binds on ganodermal manganese dependent peroxidase with Arg 69, Phe 72, Leu 192 common at their binding sites (Table 5 and figure 4). It was also observed that catechin binds to the same site that guaiacol binds on ganodermal laccase with Ala 80, His 111, Leu 112, Pro 347 common at their binding sites while flavanone binds to a different site (Table 5 and Figure 5).

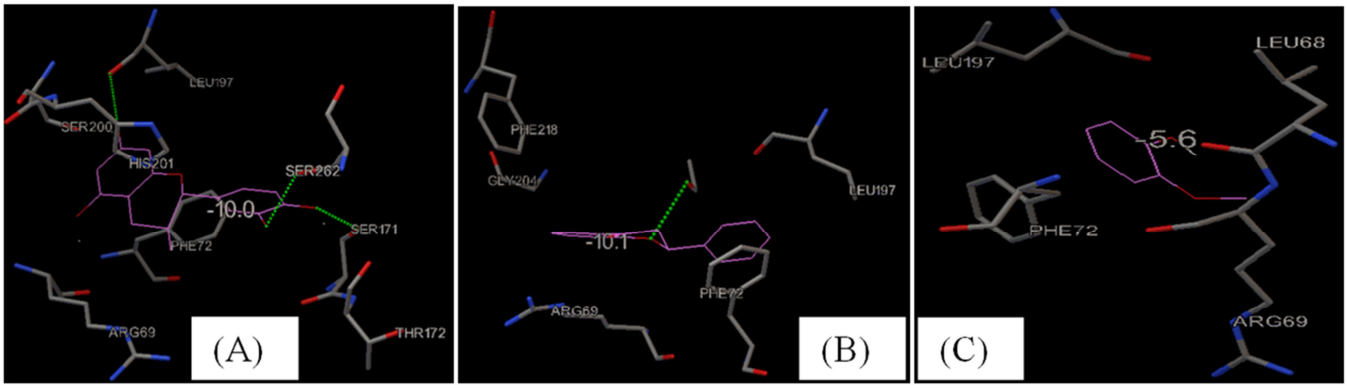

Figure 4. *Binding site of catechin (A) Flavanone (B) and Guaiacol (C) for Manganese peroxidase from Ganodema lucidium.*

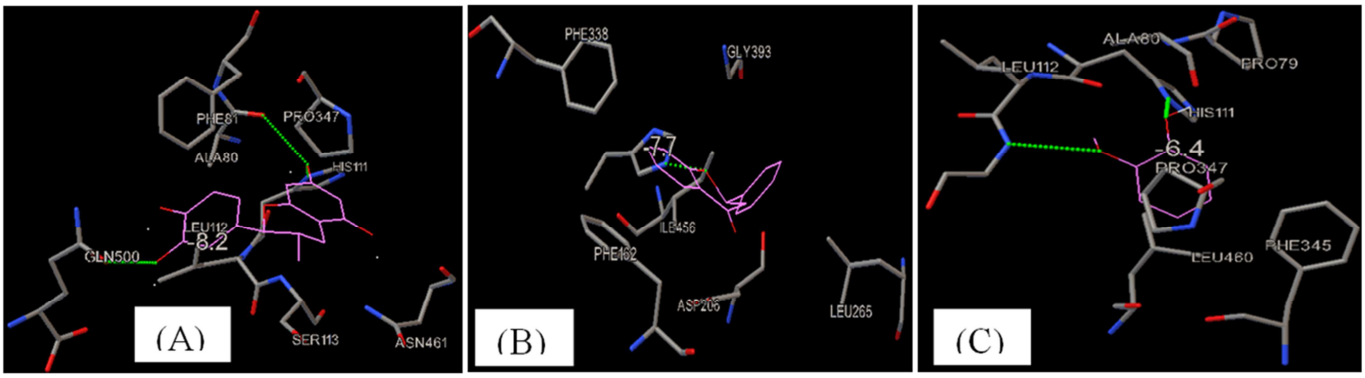

Figure 5. *Binding site of catechin (A) Flavanone (B) and Guaiacol (C) for Laccase from Ganodema lucidium.*

Table 5. *Amino acid residues at the binding sites of catechin, flavanone and quaiacol on model of Manganese peroxidase and Laccase from G lucidium.*

Laccase			Manganese peroxidase		
Catechin	**Flavanone**	**Guaiacol**	**Catechin**	**Flavanone**	**Guaiacol**
Ala 80	Phe 162	Pro 79	Arg 69	Arg 69	Leu 68
Phe 81	Asp 206	Ala 80	Phe 72	Phe 72	Arg 69
His 111	Leu 265	His 111	Ser 171	Leu 197	Phe 72
Leu 112	Phe 338	Leu 112	Thr 172	Gly 204	Leu 197
Ser 113	Gly 393	Phe 345	Leu 197	Phe 218	
Pro 347	Ile 456	Pro 347	Ser 200		
Asn 461		Leu 460	His 201		
Gln 500			Ser 262		

Laccase and manganese peroxidase are very important enzyme to *G. lucidium* because they are responsible for the degradation of lignin into low-molecular-weight compounds that can be assimilated for fungi nutrition. Therefore, stem rot disease in *E. guineensis* could be controlled by secondary metabolites that can competitively inhibit ganodermal laccase and manganese peroxidase. It has been reported elsewhere [15] that endophytic bacteria *Burkholderia gladioli pv. Agaricicola* produces flavanone.

4. Conclusion

Eight compounds showed better binding affinities for ganodermal manganese peroxidase and laccase when compared with lignin model compound (guaiacol). Catechin and flavanone are lead secondary metabolites with biocontrol potential against *G. lucidium*. Catechin and flavanone bind to the same site that guaiacol binds on ganodermal manganese dependent peroxidase and laccase while flavanone binds to a different site on laccase. Stem rot disease in *E. guineensis* could be controlled with endophytic bacteria which produces catechin and/or flavanone. Although, the *in silico* investigations show promising results but there is need for molecular dynamics simulation and field studies for validation. Further studies are necessary for comparing the bioactivity of naturally isolated volatile compounds with the commercial synthetic pesticides; verify the efficacy of catechin and flavanone against phytopathogenic fungi such as *G. lucidium*. This can help to control plant pathogens practically.

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